

AWARD NUMBER: W81XWH-12-1-0587

TITLE: Directing Spinal Cord Plasticity: The Impact of Stretch Therapy on Functional Recovery after Spinal Cord Injury.

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REPORT DATE: October 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE October 2014		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2013 - 29 Sep 2014	
4. TITLE AND SUBTITLE: Directing Spinal Cord Plasticity: The Impact of Stretch Therapy on Functional Recovery after Spinal Cord Injury.				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-12-1-0587	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) David S. K. Magnuson, PhD E-Mail: dsmagn01@louisville.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Louisville 2301 S 3 rd St. Louisville, KY 40208-1838				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Essentially all spinal cord injured patients receive stretching therapies beginning within the first few weeks post-injury. Despite this fact, almost nothing is known about how stretching might influence the neural circuitry in the spinal cord that is responsible for controlling the motor and locomotor activities of the legs. Recently, while studying activity-based rehabilitation in a rat model of spinal cord injury, we observed that stretching actually worsened locomotor recovery. The goal of this project is to investigate how the timing and intensity of a stretch-based therapy influences locomotor recovery after moderate and severe spinal cord injuries. In this, the first year of this award, we have found that stretching negatively influences locomotor function in animals with both acute (within days) and chronic (after 3 months) spinal cord injuries. We have also determined that stretching for short periods of time (4-5 weeks) allows substantial recovery to occur once stretching is stopped, and both acute and chronic animals show a similar time course of recovery. Finally, in very preliminary studies, we have found that the torque being applied during stretching of the rat hindlimb is roughly similar to that applied to human lower extremities relative to body weight.					
15. SUBJECT TERMS Spinal cord injury, stretching, physical therapy, rehabilitation, locomotor recovery					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER (include area code)

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Annual Report:

SC110169 – “Directing Spinal Cord Plasticity: The Impact of Stretch Therapy on Functional Recovery after Spinal Cord Injury”.

Principle Investigator: David S. K. Magnuson, PhD. University of Louisville.

Introduction:

This research focuses on the impact of stretching (physical therapy maneuvers involving force or torque applied to specific muscle groups) on functional recovery after spinal cord injury in a rat model. We have undertaken these studies because of an observation we made a few years ago during a study where the hindlimbs of rats with spinal cord injuries were being immobilized in a wheelchair. We found that immobilization dramatically influenced locomotor recovery, presumably, by reducing the sensory input associated with movement (Caudle et al., 2011). In that study we employed a stretching procedure designed to prevent reductions in joint range-of-motion. The stretching didn't prevent contractures, but it did have a negative impact on locomotor recovery. Thus, in the current study, specific Aim (SA) 1 focuses on the timing of stretching relative to the injury and whether or not there is a window of susceptibility to a stretching-based therapy. SA2 focuses on the pattern and forces of the actual stretching protocol and if the negative influence of stretching is due primarily to the length of each maneuver or to the forces applied during stretch. An overarching goal in the project is to develop a computer model of the stretching to allow direct comparison of rat and human.

Key Words: spinal cord injury, locomotor recovery, physical therapy, muscle stretch, joint range-of-motion, rat.

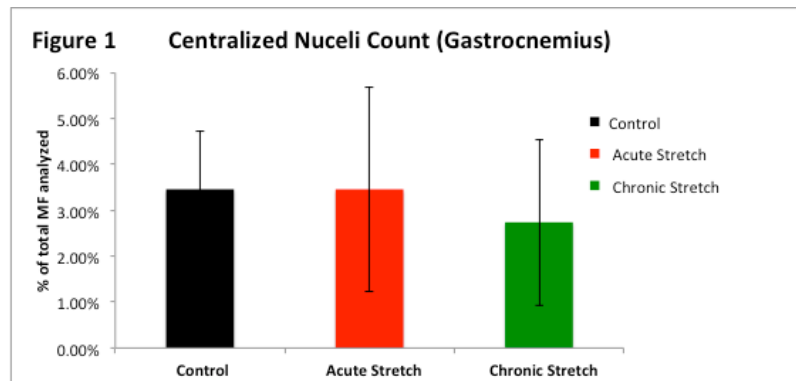
Overall Project Summary:

In this, the second year of this project, we have accomplished substantial components of Tasks 1, 2, 3 and 5 as described in the original Statement of Work. This has involved aspects of Specific Aims 1 and 2 as follows.

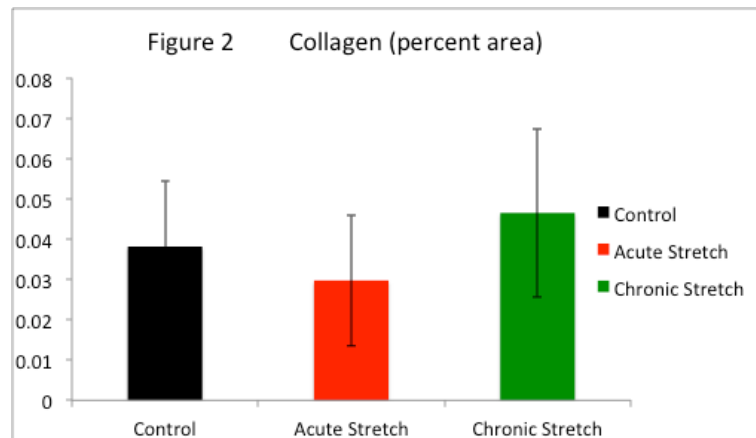
Muscle Damage During Stretching.

A key determination for the translation of our findings to the human (clinical) situation is whether or not we are damaging hindlimb muscles when we stretch the animals after a spinal cord injury. We have used two different standard and well-accepted approaches to assess muscles for damage. The first is staining sections of muscle for centralized nuclei and the second is staining for collagen deposition. Muscle cells (myocytes) are multinucleated super-cells and normally the nuclei are found along the edge of the muscle fiber squeezed between the contractile proteins and cell membrane. Even mildly injured muscles show an increased proportion of nuclei that appear in the center of the muscle fiber (centralized). Hindlimb muscles from our “acute/chronic” study completed and reported on last year, have been processed for centralized nuclei and we found that stretched and unstretched (control) animals have similar, and normal, low numbers of centralized nuclei (Figure 1).

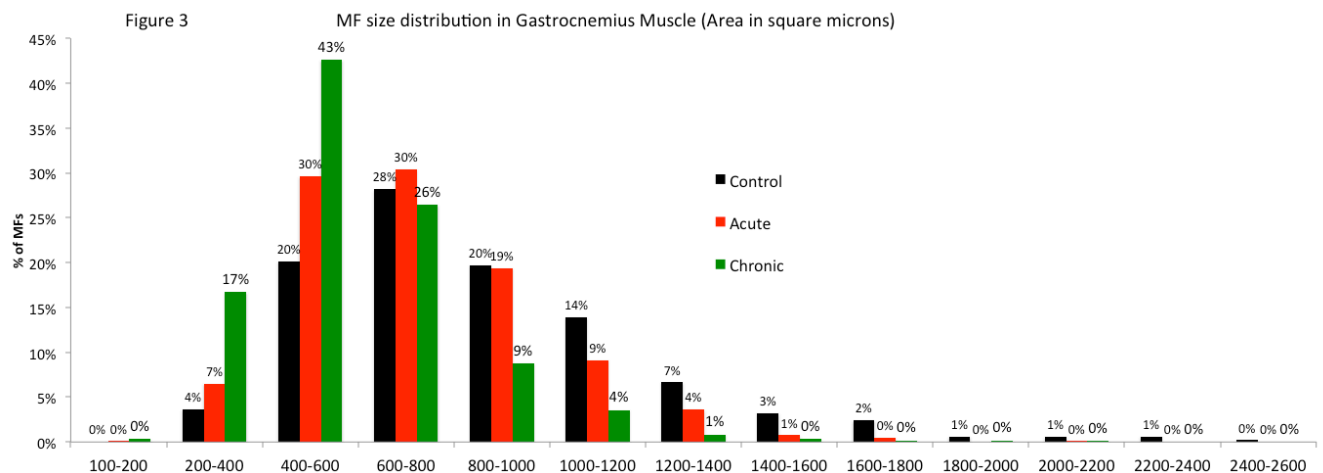
Skeletal muscle, like those in the rat hindlimb, have a small amount of connective tissue, composed largely of collagen, that is intermingled with the muscle fibers, with slightly larger amounts being in and around the major blood vessels and between



the muscle fascicles. When a muscle is injured, part of the process of recovery involves an increasing deposition of connective tissue (collagen) between the fascicles, sort of like a mild scarring. The amount of collagen can easily be quantified using Masson's Trichrome stain and an image analysis system (we use Image J). The hindlimb muscles from the rats involved in our acute/chronic stretching study completed last year have been processed using Masson's Trichrome stain and then collagen has been quantified using a standardized Image J routine to count pixels in cross section to estimate the area of collagen in each section. As can be seen in Figure 2, using the gastrocnemius muscle as an example, there is no difference between stretched and unstretched hindlimb muscles. We assessed the tibialis anterior, an ankle flexor, and the gastrocnemius muscles, and ankle extensor, bilaterally, and found no differences between stretched and unstretched groups.



In addition to centralized nuclei and collagen deposition, we also examined muscle fiber size distribution. This is done simply by sectioning and staining the muscle and then randomly selecting fibers to measure (in square microns). Normally, both rats and humans lose muscle mass after a spinal cord injury, usually because of disuse, and this involves a drop in fiber numbers, but more importantly an decrease in fiber diameter indicating that each muscle fiber is smaller, weaker and has lost critical contractile proteins. This analysis is still ongoing, but the distribution of fiber sizes shown in Figure 3 suggest that stretching is having a influence on fiber size, perhaps by reducing the use of the hindlimbs during in-cage activity and that reduction in activity is leading to increased muscle atrophy. Interestingly, there is a clinical phenomenon that stretching can lead to muscle fiber hypertrophy, but that doesn't appear to be happening in our studies.



Kinematics, Kinetics and EMG Responses to Stretching

A second, and perhaps even more important aspect of this project that will allow us to translate our findings to the clinical situation is to characterize the response of the nervous system to the actual stretch and to quantify the stretch itself. We are currently 4 weeks into an 8 week study designed to accomplish these goals. This is a very complex, team performed study involving 8

animals divided into two groups. One group received telemetry devices (Data Sciences International) that allows us to record from two hindlimb muscles and to acquire that data wirelessly. The other 4 animals are controls so that we can know if the transmitters and intramuscular wires influences function and functional recovery. After acquiring baseline data from the animals uninjured, including data from stretching when the animals were anesthetized, all the animals were given moderate T9 contusion spinal cord injuries. We have then collected data from all 8 animals twice a week. The data collection involves the kinematics of walking (how the limbs move while the animals walk along a plexiglass tank), the kinematics of stretching (the position and movement of the limbs during the stretch), the forces being applied during the stretch (using the “force glove) based on force sensitive resistors, as reported previously, and finally, the EMG or electromyogram from the hamstring and quadriceps muscles recorded during the stretch itself. So far we have only stretched the contralateral limb, the limb not implanted with EMG wires, however, later in the study we will record activity during the stretching of both the contralateral and ipsilateral (implanted) limb. None of the data has been analyzed sufficiently to allow it to be presented here, but I can describe several very important observations that we have made so far.

Observations:

1. Stretching of one hindlimb results in at least three distinct EMG patterns in the contralateral hindlimb. Two of these patterns are related to movement (air stepping and a “clonus” like response). One appears to be unrelated to movement but involves high frequency but low amplitude co-activation of antagonist muscles.
2. The highest levels of force during stretching sometimes exceed 400g (well beyond body weight), but these peaks of force occur during the movements described above (air stepping or clonus) or other related movements that can feel like the animal is fighting the stretch.
3. Stretching one limb has a temporary negative impact on hindlimb function bilaterally (few hours), and a slightly longer negative impact on hindlimb function ipsilaterally (stretched limb, up to a day).
4. We believe that we will be able to identify an “EMG signature” of stretching and, in studies planned for the next quarter, an “EMG signature” of the negative impact of stretching that can then be used in clinical studies to determine if stretching in patients is having a negative impact on function that has been heretofore unrecognized.

Key Research Accomplishments.

The results described above are very significant steps towards completing the proposed studies and will provide critical information as we move forward. The primary research accomplishments overall for this project are:

1. The forces being applied during stretching of the rat are roughly similar to those being applied to humans clinically, based on a body-weight comparison.
2. That daily stretching, whether applied in the acute or chronic phase, can be devastating to motor function, and in the light of our previous work, if stretching is continued for 8 weeks or more, the deficits may be long-lasting.
3. That deficits resulting from both acute and chronic stretching can be viewed as temporary if the stretching is only done for a few weeks. This finding has to be viewed with the caveat that rats are more active than people (post-injury) and that this activity may be what allows them to recover after several weeks of stretching.
4. Stretching of one hindlimb is strongly influencing spinal cord circuitry bilaterally, resulting in several distinct patterns of muscle activation in the limb not being stretched.

Some of this activation is via circuits involved in movement, seen as airstepping and clonus, and some of this activation is via other circuits or is more general resulting in high-frequency, low amplitude co-activation patterns that are clearly abnormal.

5. Peak forces during stretch are the result of muscle activation, not the force being applied by the therapist.
6. Despite the peak forces of more than 400g, neither daily acute nor daily chronic stretching (for 4-5 weeks) resulted in muscle damage as assessed by counting centralized nuclei and quantification of collagen deposition.

These findings are extremely significant and will be strengthened as all the data is analyzed and we begin to relate the details of the forces, kinematics (joint angles) and EMG patterns for each of the muscle groups..

Conclusion:

Our results so far are extremely important because they validate and extend what we found previously and begin to gather the information we need to move towards clinical translation. They demonstrate that the forces we are using while stretching the rats are not causing overt muscle damage and that the peak forces are occurring in response to nervous system activation, and not the stretching force itself. We are building up a picture of how the spinal cord is responding to the afferent input caused by the stretching and that at least some of this response involves co-activation at high-frequency and low amplitude. There continues to be solid rationale for proceeding with the remaining experiments as described in the original proposal. None of our results are pointing towards the development of a product, but will lead to the suggestion that our current stretching practices in the clinic will need to change. Our remaining experiments will help to illustrate how those practices should change.

Publications, abstracts and presentations:

This project has resulted in one full-length publication this year. The full reference is included below (Caudle et al., 2014). This paper reports on a study that was initiated prior to grant submission, but was completed after the grant was awarded. This project has also resulted in an abstract/poster presentation at the National Neurotrauma Society meeting in San Francisco in August (full reference shown below).

Inventions, Patents and Licenses: Nothing to report.

Reportable Outcomes: Nothing to report.

Other Achievements: Nothing to report.

References:

Caudle KL, Atkinson DA, Brown EH, Donaldson K, Seibt E, Chea T, Smith E, Chung K, Shum-Siu A, Cron C, Magnuson DSK. Hindlimb stretching alters locomotor function post-spinal cord injury in the adult rat. *Neurorehab and Neural Repair* (in press).

Keller AV, Shum-Siu A, Wainwright GN, Seibt S and Magnuson DSK. Hindlimb muscle stretch reduces locomotor function after a Spinal Cord Injury: Implications for Physical Therapy and Rehabilitation. National Neurotrauma Society Meeting, August 2014. San Francisco, CA.

Appendix: Link to Caudle et al., 2014. Neurorehab and Neural Repair.

<http://nnr.sagepub.com/content/early/2014/08/07/1545968314543500.long>